RUHR-UNIVERSITÄT BOCHUM

Structure, pathogenesis, working methods and clinical diagnostics of viruses

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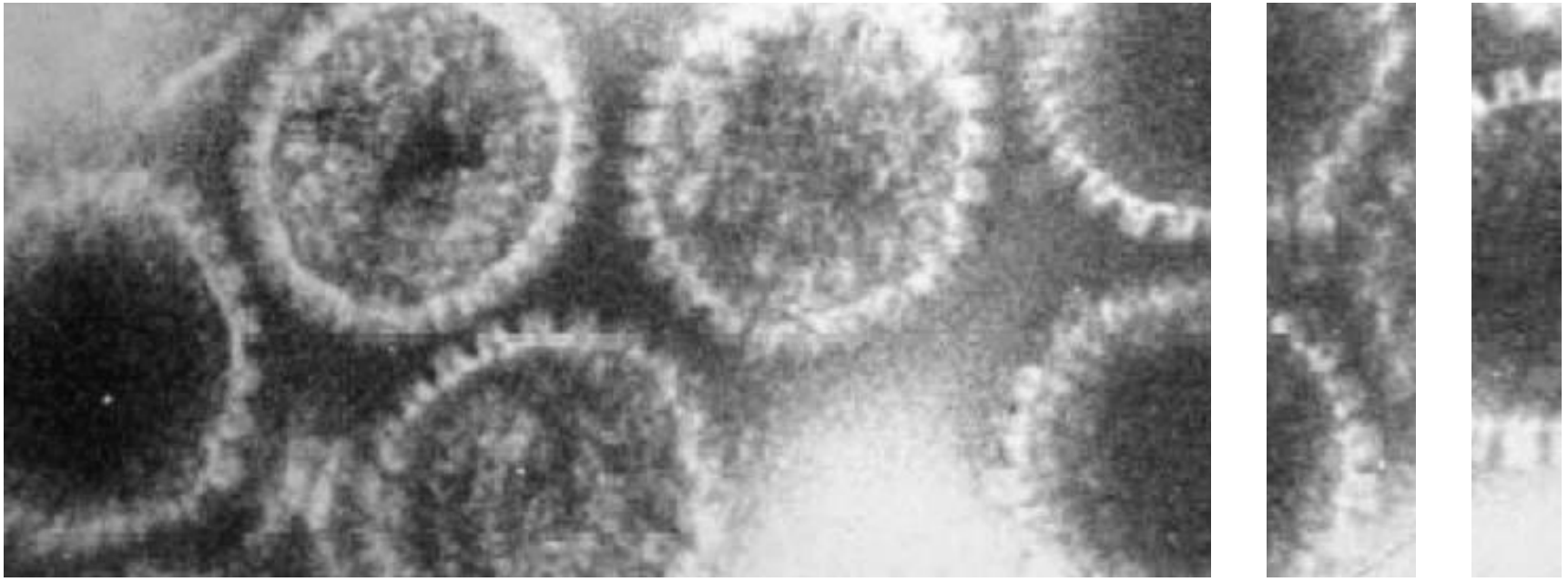


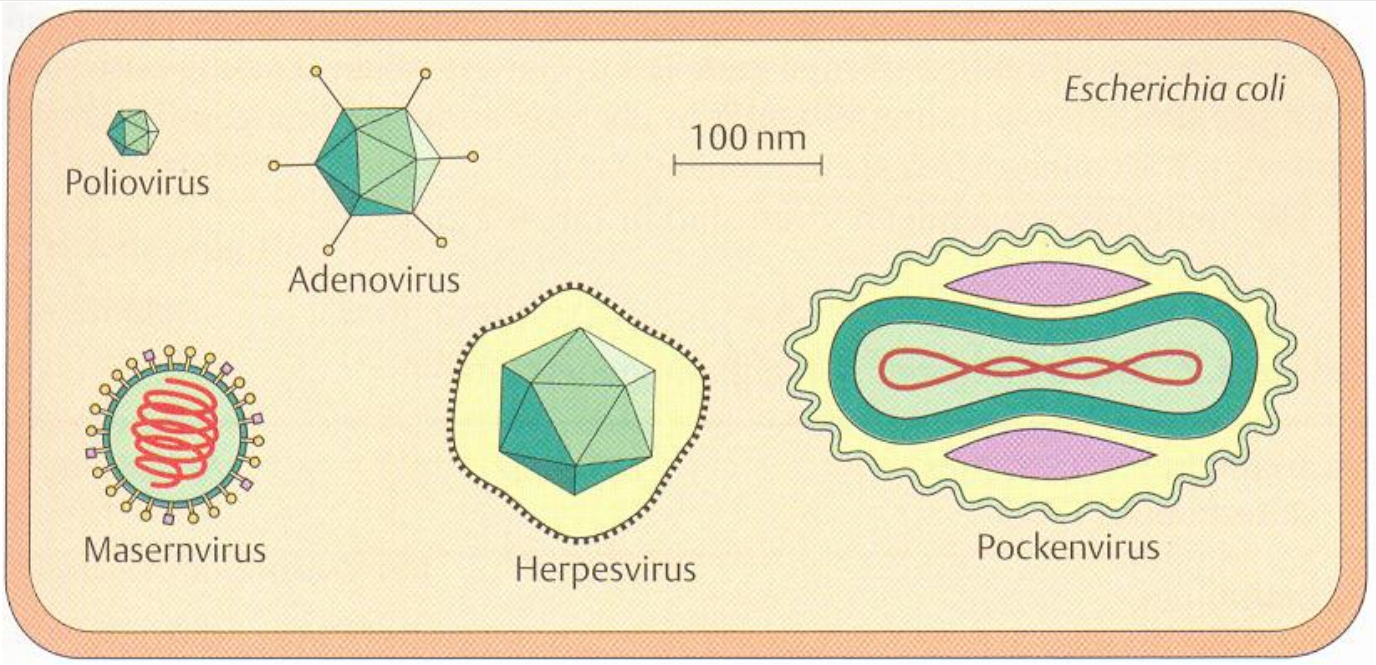
Henle-Koch-Postulates (1882)

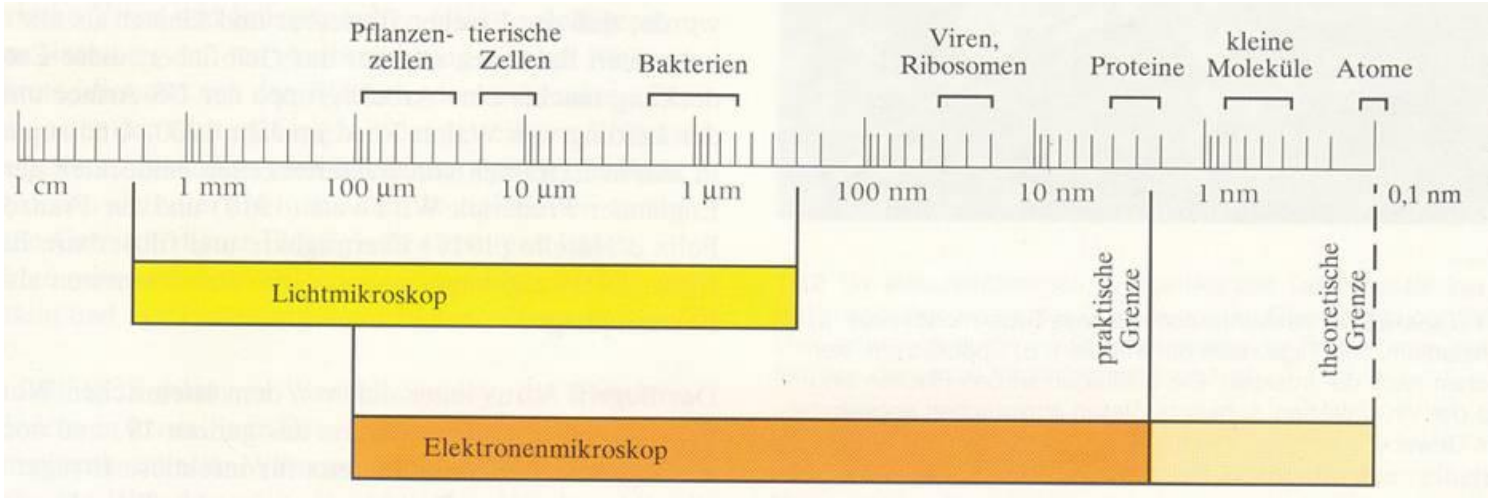
1. The microorganism must be found in abundance in all organisms suffering from the disease
2. The microorganism must be isolated from a diseased organism and grown in culture
3. The cultured microorganism should cause disease when introduced into a healthy organism
4. The microorganism must be reisolated from the inoculated, diseased experimental host

History

- Smallpox vaccine (Edward Jenner, 1796)
- Vaccination against rabbies with brain extract from infected rabbits (Louis Pasteur, 1885)
- Differentiation from bacteria
  - 1892 Dimitri Iwanowski: Infectous agens of mosaic disease can be ultrafiltrated (< 200 nm)
  - M. W. Beijerinck: Concept of a self replicating „liquid“ agens
  - 1898 F. Loeffler & P. Frosch: First proof for an animal virus (foot and mouth disease)
- Development of electron microscopy (Ruska, 1931)
- Virus isolation in hen‘s eggs (Goodpasture, 1931)
- Cristallization of Viruses (Stanley, 1935)
- Virus isolation in cell culture (Enders, Robbins, Weller, 1949)

Size comparison viruses/bacteria

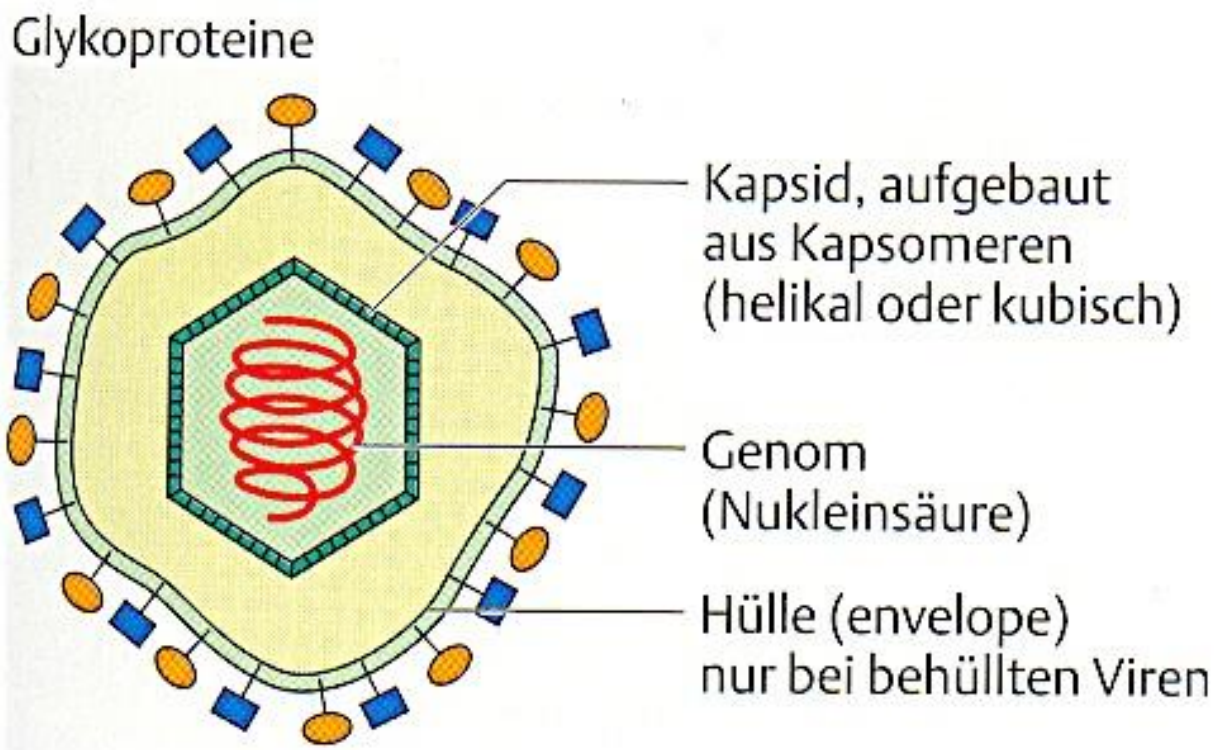




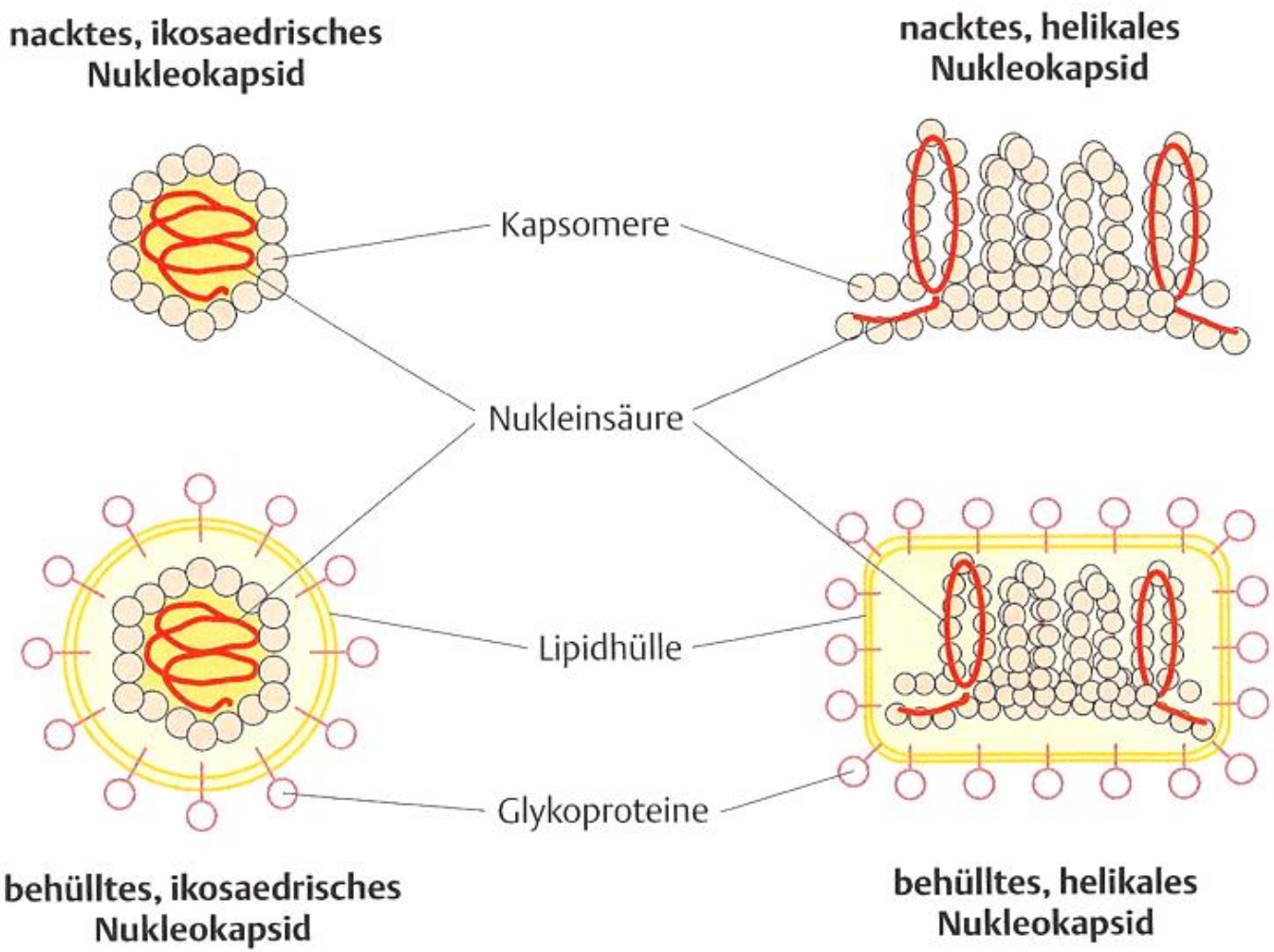
Characteristic properties of viruses

- Size: 25 nm (picorna viruses) – 400 nm (Orthopox)
- Genome: DNA or RNA (never both)
- Nucleic acid can be single stranded or double stranded, linear, segmented, circular
- Genome sizes:
  - RNA-viruses 1.7 kb (Deltavirus) – 24 kb (Reoviruses);
  - DNA-viruses 1.7 kb (Circoviuses) – 250 kb (Poxviruses)
- Replication is dependent on cellular metabolism

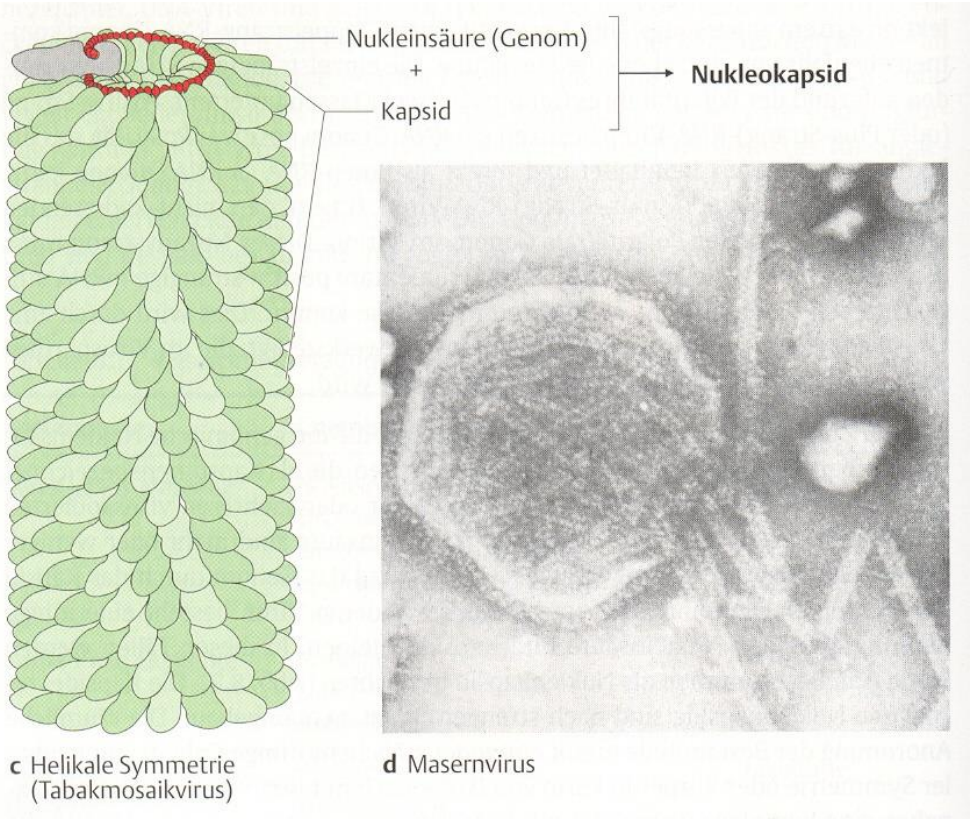
Components of virus particles (virions)



General structures of viruses

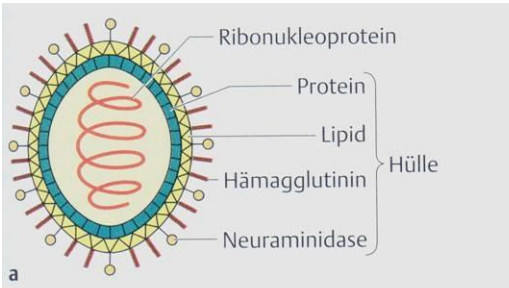


Viruses with a helical symmetry



c Helikale Symmetrie (Tabakmosaikvirus)

d Masernvirus



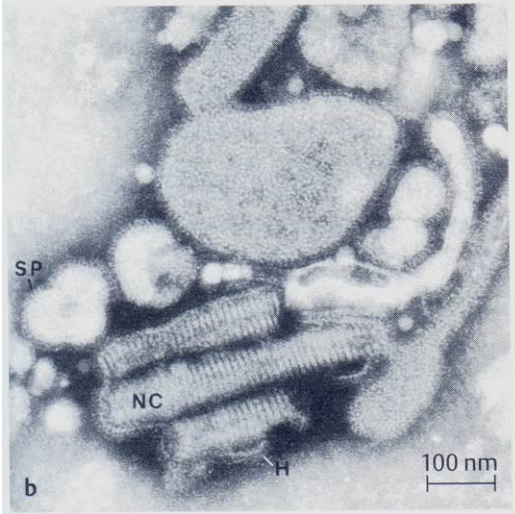
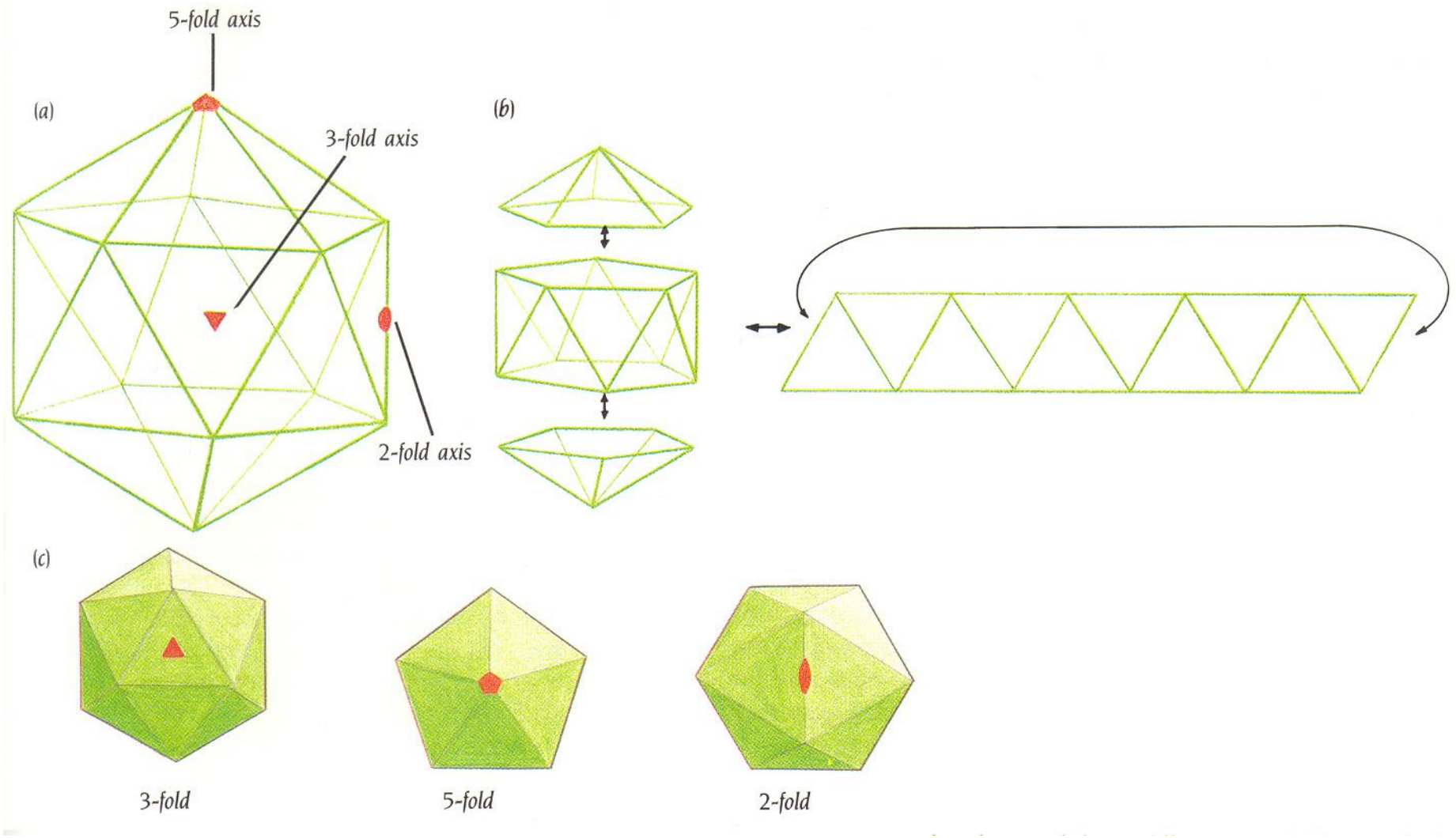
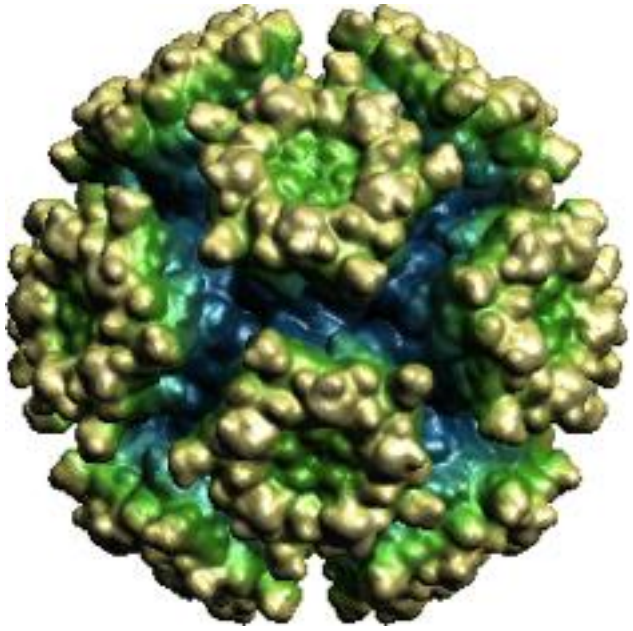


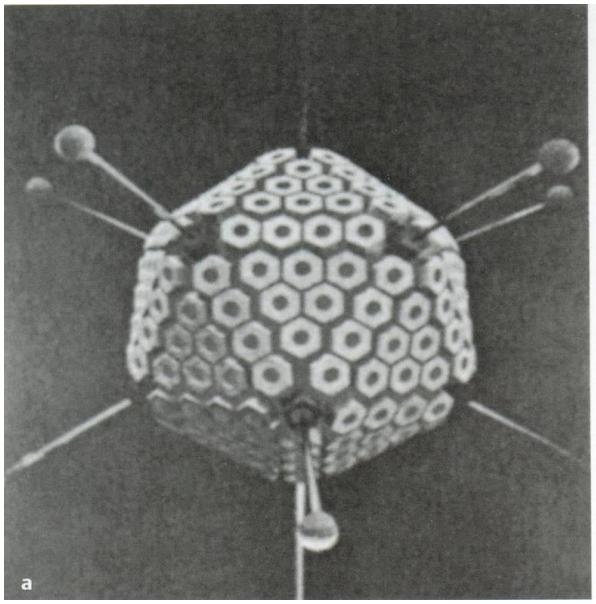
Abb. 7.3 **a** Schematischer Aufbau am Beispiel der Myxoviren. **b** Elektronenmikroskopische Aufnahme von Influenzaviren: Die Ribonukleoprotein-Spirale (Nukleokapsid = NC) ist innerhalb der teilweise abgelösten Hülle (H) sichtbar. SP = Spikes.

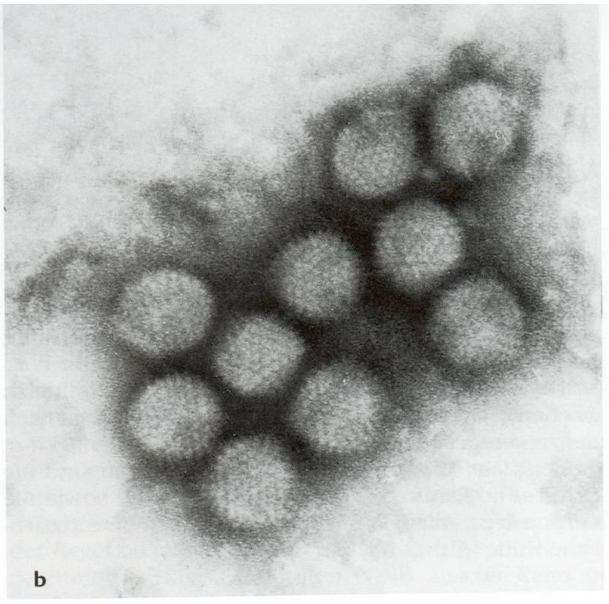
Rotational symmetry of icosaeders



Adenovirus, example for an icosahedral capsid

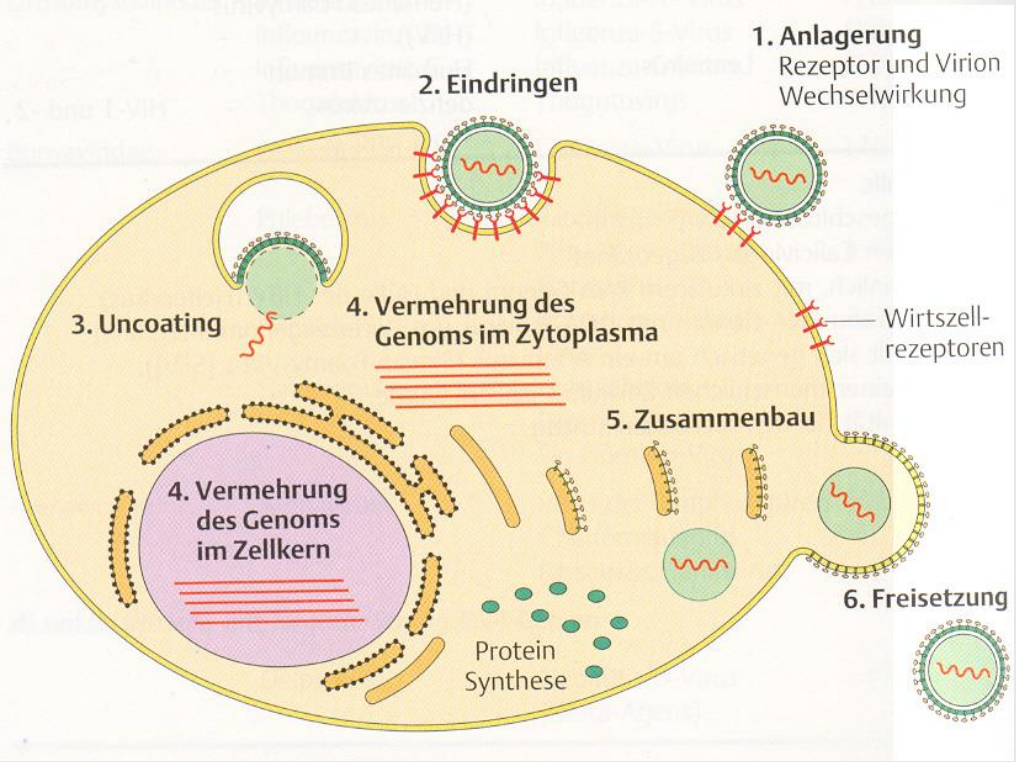






Viral replication cycle

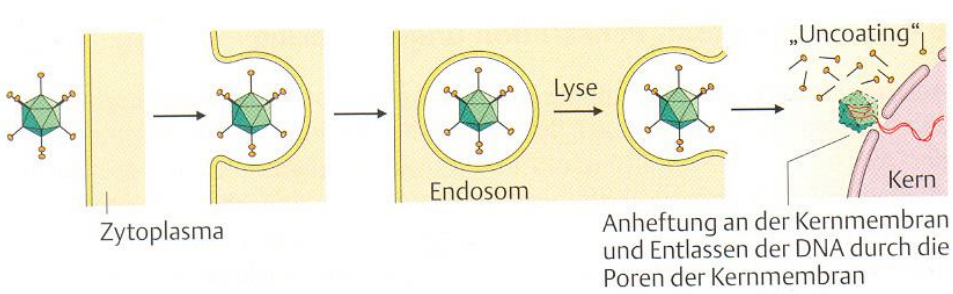
- Attachment
- Penetration
- Release of viral nucleic acid
- mRNA synthesis (not for ss-(+)-RNA viruses)
- Protein synthesis
- Replication of nucleic acid
- Assembly and maturation
- Release from host cell

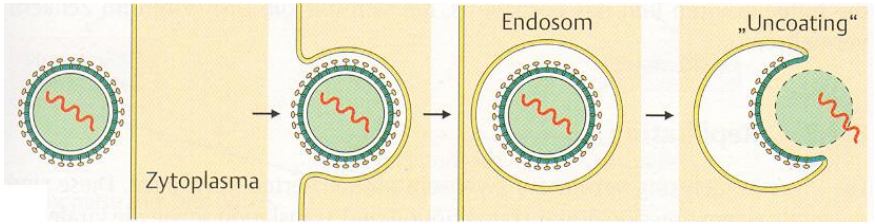


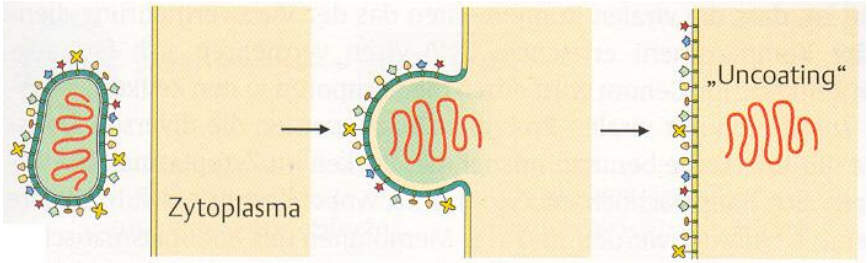
Cellular receptors

• ICAM1	Rhinoviren
• Ig-Superfamily	Polioviren
• Acetylcholin receptor	Rabiesvirus
• Phosphatidylserin	VSV
• Sialinic acid structures	Influenza A
• CD4 (CCR5, CXCR4, CCR2)	HIV
• Blutgruppenantigen P	PV B19
• Complement receptor 2	EBV
• **E**pidermal **g**rowth **f**actor **r**eceptor	Vacciniavirus

Mechanisms of attachment, penetration and uncoating





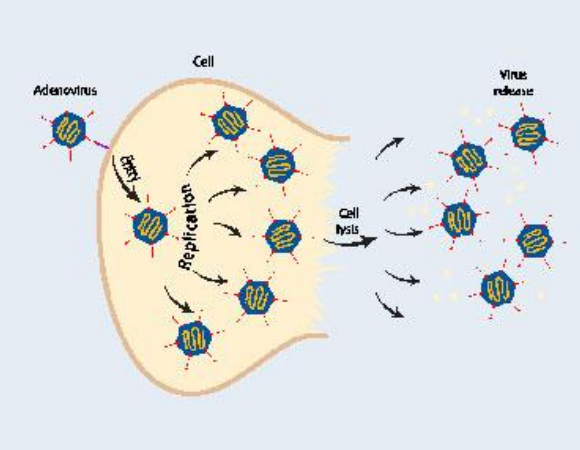


Genome replication

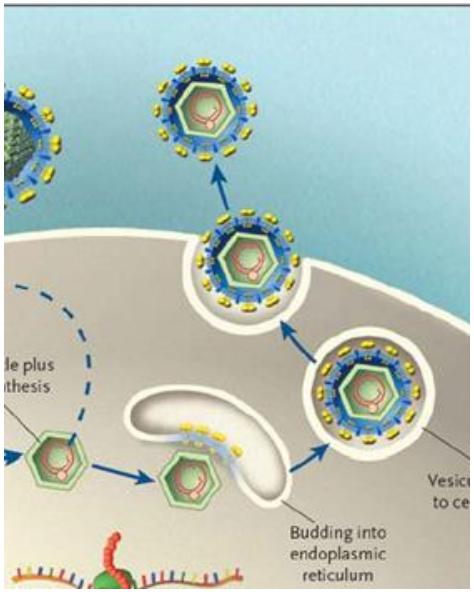
- DNA viruses
  - ss-DNA: Cellular Polymerases (ds intermediate)
  - ds-DNA: Replication mediated by cellular (Polyomaviruses) or viral enzymes (Herpesviruses)
- RNA viruses
  - (+) ss-RNA: direct translation (polyprotein of Picornaviruses)
  - (-) ss-RNA: polymerase in virion; complementary RNA is used as mRNA + 1 contiuous as template for new genomes
  - ds-RNA: polymerase in virion (Rotaviruses); produces mRNA from (-)-strand
  - Retroviruses: (+) ss-RNA; reverse transcriptase in virion (ds-DNA intermediate)

Release of virions

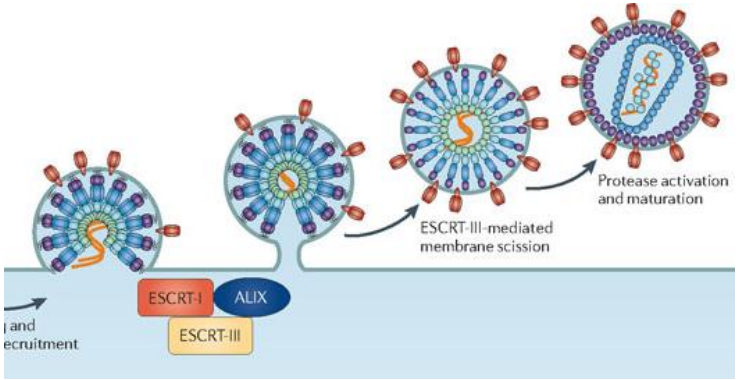
Lysis



Exocytosis



Budding



Virus taxonomy

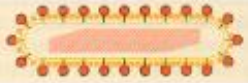
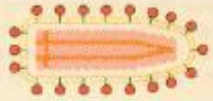
- Order: e.g. Herpesvirales
  - Family: e.g. Herpesviridae
    - Subfamily: e.g. Alphaherpesvirinae
      - Genus: e.g. Simplexvirus
        - Species: e.g. Human Herpesvirus 1 (HHV1 = HSV1)
          - Strain: e.g. HFEM

Citeria for virus taxonomy

- Nucleic acid: DNA/RNA, ss/ds, polarity, linear/circular, segmented/unsegmented, size
- Replication
- Proteins: number, properties, functions
- Virion: size, morphology, physical properties
- Biological properties

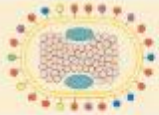
Virus families

a RNA-Viren

Familie	Gattung	wichtige Arten	Eigenschaften	schematischer Bauplan
Picornaviridae	Enterovirus Hepatovirus Rhinovirus Cardiovirus	Polio-, Echo-, Coxsackie-Viren Hepatitis-A-Virus Rhinovirus 1–117 Mengo-, EMC-Virus	Größe: 24–30 nm Kapsid: kubisch Hülle: nein Genom: RNA,ss (+)	
Caliciviridae	Norovirus	Norwalkvirus	Größe: 27–30 nm Kapsid: kubisch Hülle: nein Genom: RNA,ss (+)	
Hepeviridae	Hepevirus	Hepatitis-E-Virus	Größe: 33 nm Kapsid: kubisch Hülle: nein Genom: RNA,ss (+)	
Reoviridae	Coltivirus Reovirus Rotavirus	Colorado-Zeckenfieber-Virus Reovirus 1–3 Rotaviren	Größe: 60–80 nm Kapsid: kubisch Hülle: nein Genom: RNA,ds segmentiert	
Coronaviridae	Coronavirus	Coronaviren	Größe: 80–220 nm Kapsid: helikal Hülle: ja Genom: RNA,ss (+)	
Togaviridae	Alphavirus Rubivirus	Sindbis-Virus Rötelvirus	Größe: 60–70 nm Kapsid: kubisch Hülle: ja Genom: RNA,ss (+)	
Flaviviridae	Flavivirus Hepacivirus	Gelbfiebervirus FSME-Virus Hepatitis-C-Virus	Größe: 40 nm Kapsid: kubisch Hülle: ja Genom: RNA,ss (+)	
Arenaviridae	Arenavirus	LCM-, Lassa-Virus	Größe: 50–300 nm Kapsid: Komplex Hülle: ja Genom: RNA,ss (+/–) segmentiert	
Filoviridae	Marburgvirus Ebolavirus	Lake-Viktoria-Marburg-Virus Zaire-Virus	Größe: 80x Filament (nm) Kapsid: helikal Hülle: ja Genom: RNA,ss (–)	
Bunyaviridae	Orthobunyavirus Nairovirus Phlebovirus Hantavirus	California-Enzephalitis-Virus Krim-Kongo-Virus Phlebotomus-Fieber-Virus Hantaan-Virus	Größe: 100 nm Kapsid: helikal Hülle: ja Genom: RNA,ss (–) segmentiert	
Orthomyxoviridae	Influenzavirus	Influenza-A-, -B-, -C-Virus	Größe: 80–120 nm Kapsid: helikal Hülle: ja Genom: RNA,ss (–) segmentiert	
Paramyxoviridae	Pneumovirus Paramyxovirus Rubulavirus Morbillivirus	Respiratory syncytial virus Parainfluenzavirus 1 und 3 Mumpsvirus Masernvirus	Größe: 150–300 nm Kapsid: helikal Hülle: ja Genom: RNA,ss (–)	
Rhabdoviridae	Lyssavirus	Tollwutvirus	Größe: 60–180 nm Kapsid: helikal Hülle: ja Genom: RNA,ss (–)	

Familie	Gattung	wichtige Arten	Eigenschaften	schematischer Bauplan
Retroviridae	Deltaretrovirus Lentivirus	HTLV I und II HIV 1 und 2	Größe: 100 nm Kapsid: Komplex Hülle: ja Genom: RNA,ss (+) segmentiert	

b DNA-Viren

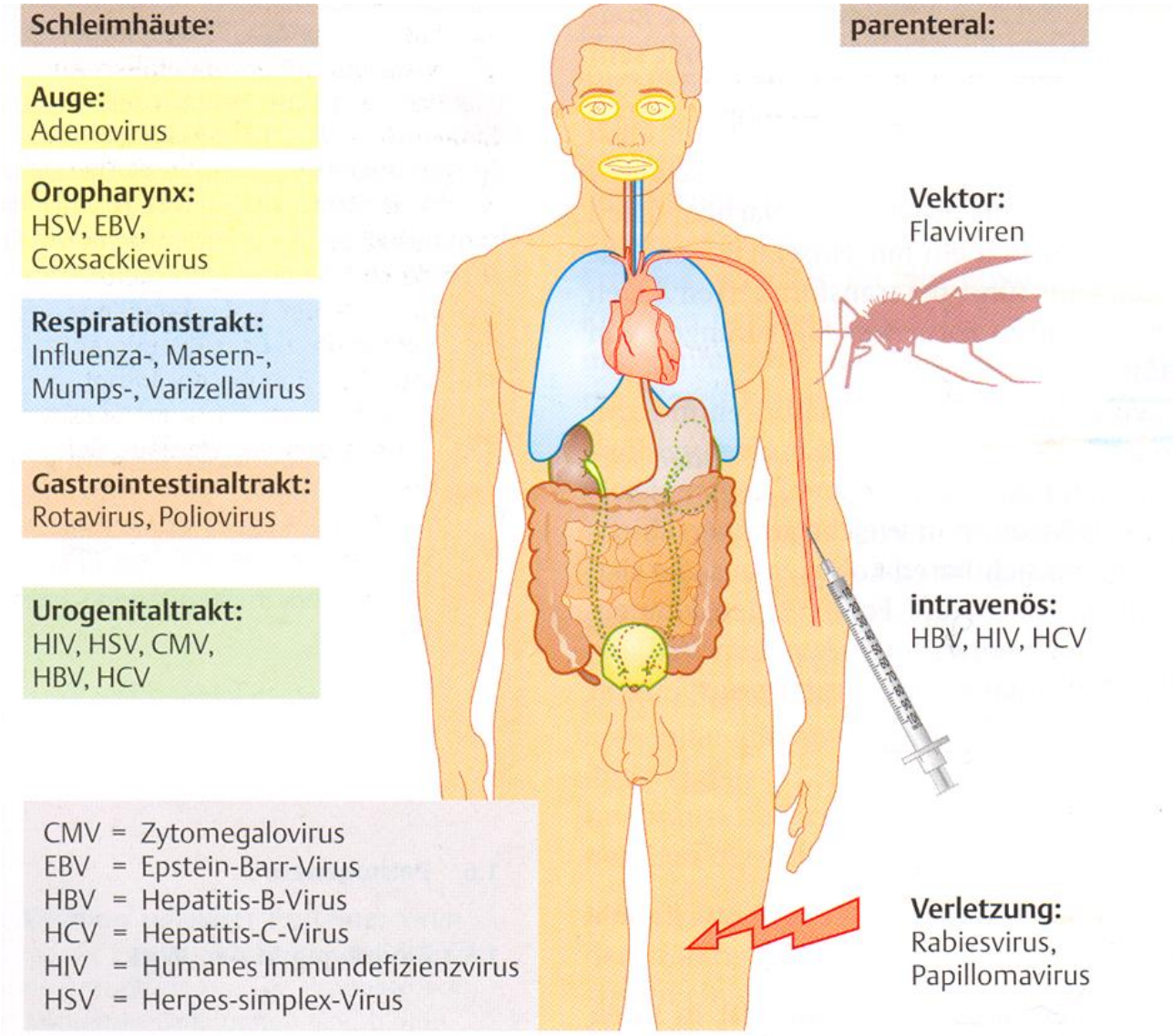
Familie	Gattung	wichtige Arten	Eigenschaften	schematischer Bauplan
Herpesviridae	Simplexvirus Varicellovirus Cytomegalovirus Roseolovirus Lymphokryptovirus	Herpes-simplex-Virus Varizellen-/Zoster-Virus Zytomegalovirus Humanes Herpesvirus 6 Epstein-Barr-Virus	Größe: 100/200 nm Kapsid: kubisch Hülle: ja Genom: DNA, ds	
Papillomaviridae	Papillomavirus	Warzenvirus	Größe: 55/45 nm Kapsid: kubisch Hülle: nein Genom: DNA, ds	
Polyomaviridae	Polyomavirus	BKV, JCV	Größe: 55/45 nm Kapsid: kubisch Hülle: nein Genom: DNA, ds	
Parvoviridae	Erythrovirus	Parvovirus B 19	Größe: 19–25 nm Kapsid: kubisch Hülle: nein Genom: DNA, ss	
Adenoviridae	Mastadenovirus	Adenoviren	Größe: 70–90 nm Kapsid: kubisch Hülle: nein Genom: DNA, ds	
Poxviridae	Orthopox Parapox	Variola-, Vaccinavirus Orf	Größe: 230–350 nm Kapsid: komplex Hülle: ja Genom: DNA, ds	
Hepadnaviridae	Orthohepadnavirus	Hepatitis-B-Virus	Größe: 27/42 nm Kapsid: kubisch Hülle: ja Genom: DNA, ds/ss	

Pathogenesis

Routs of transmission

- Aerogen
  - Droplets, aerosols
- Fecal-oral
  - Smear infection, contaminated surfaces/instruments
- Sexual
- Percutaneous
- Intrauterine-perinatal
- Iatrogenic
  - Transplants, blood products, personnel, contaminated instruments
- Indirect
  - Water, food

Entry of some human viruses



Progressions of viral infections

- Inapparent (asymptomatic)
- Apparent (symptomatic)

Polio	< 1 % app.
Measles	> 95 % app.
Primary HSV	5-10 % app.
Mumps	50 % app.

Damages caused by viral infections

- Primary damages
  - Feedback of virus synthesis on cellular metabolism
  - Cytopathic effects (CPE): morphologic alterations, Syncytia, apoptosis, genetic changes (chromosomal aberrations, genomic integration, malign transformation)

- Secundary damages
  - Immuno pathological processes

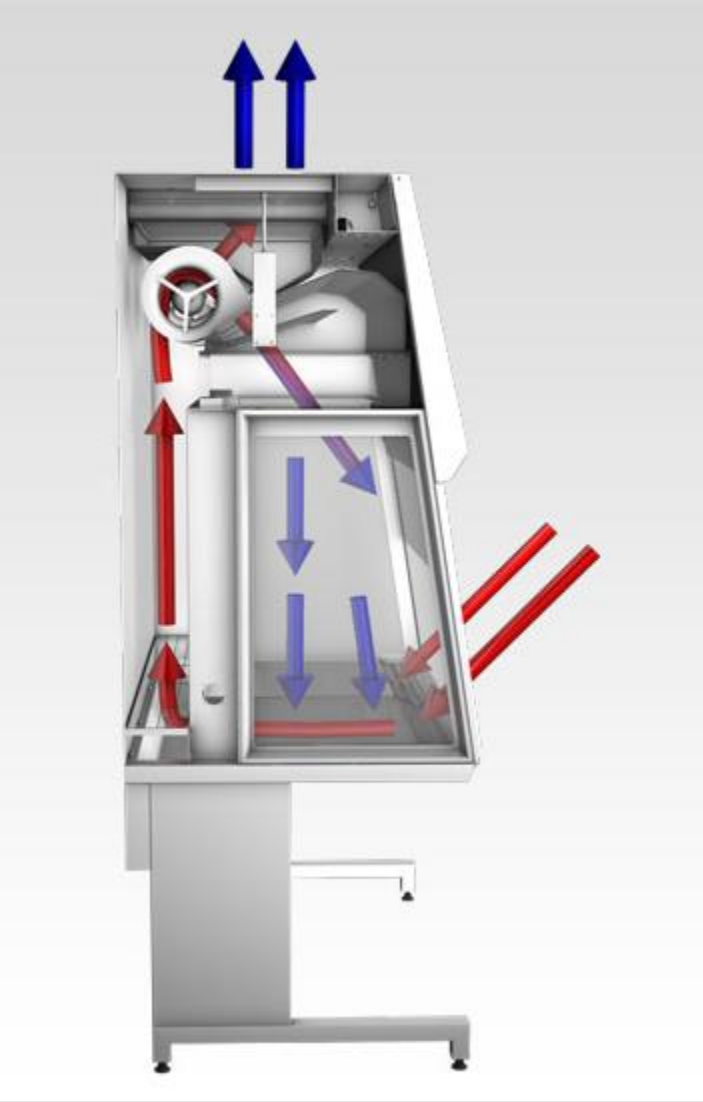
Working methods

Legal requirements for a virologic lab (Germany)

- Infektionsschutzgesetz (IfSchG)
  - Controls working with replication competent pathogens
  - Required: proof of competence
- Biostoffverordnung (BioStoffV)
  - Safety precautions for works with biological agents
- Gentechnikgesetz (GenTG)
  - Production of, working with or release of genetically modified organisms (GVO)
  - Prevention and control of hazards
- In general:
  - Microorganisms are classified into 4 biohazard level groups (R1-R4), which require appropriate safety level precautions (S1-S4) and lab equipment (L1-L4)

Basic equipment for cell culture

- Laminar-flow cabinet („hood“)
- Incubator (CO_2)
- Water bath
- Cooling centrifuge
- Inverted microscope
- Refrigerators, freezers (-20°C, -80°C)
- Cryoconservation in liquid Nitrogen
- Culture dishes and bottles
- Media
- Autoclave
- Equipment for liquid handling



Virus purification

- Separation of cellular components
  - Filtration (0.2 µm)
  - Low speed centrifugation
- Optional: concentration by PEG-precipitation
- Ultracentrifugation
  - CsCl gradient
  - Sucrose gradient/cushion

Clinical diagnostics

Clinical diagnostics of viruses

- **Direct virus detection (detects components of the virus/virion)**
  - Virus antigen
  - Virus genome
  - Complete virions
  - Secondary effects of a virus

- **Indirect detection (detects response of immune system after infection)**
  - Mostly specific antibodies (IgM, IgG)

Direct vs. Indirect detection

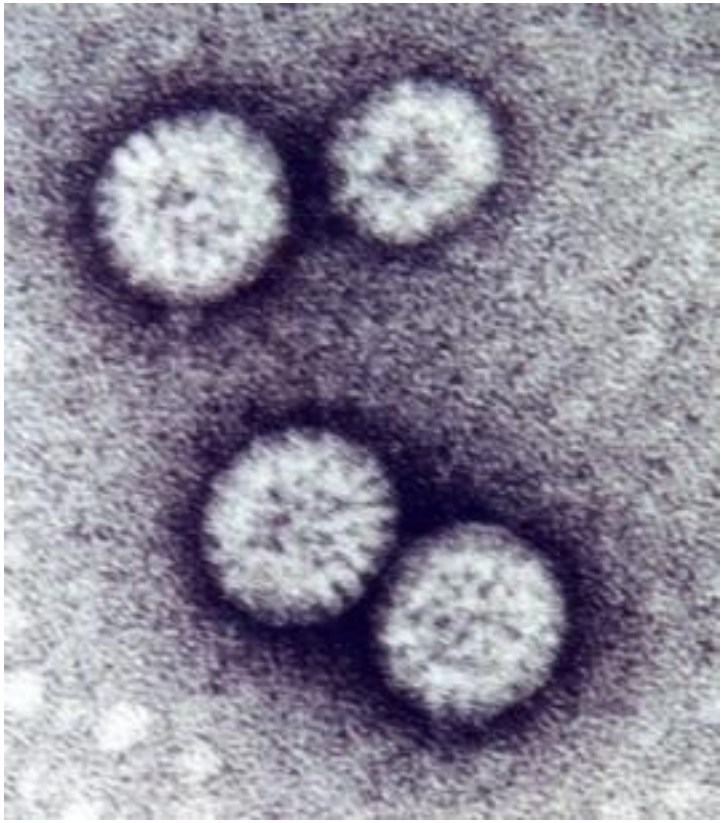
Direct

- Advantages:
  - In early phase of infection possible
  - Can identify the phase of infection
  - Monitor success of therapy
- Disadvantages:
  - Detection only in acute or chronic phase of infection

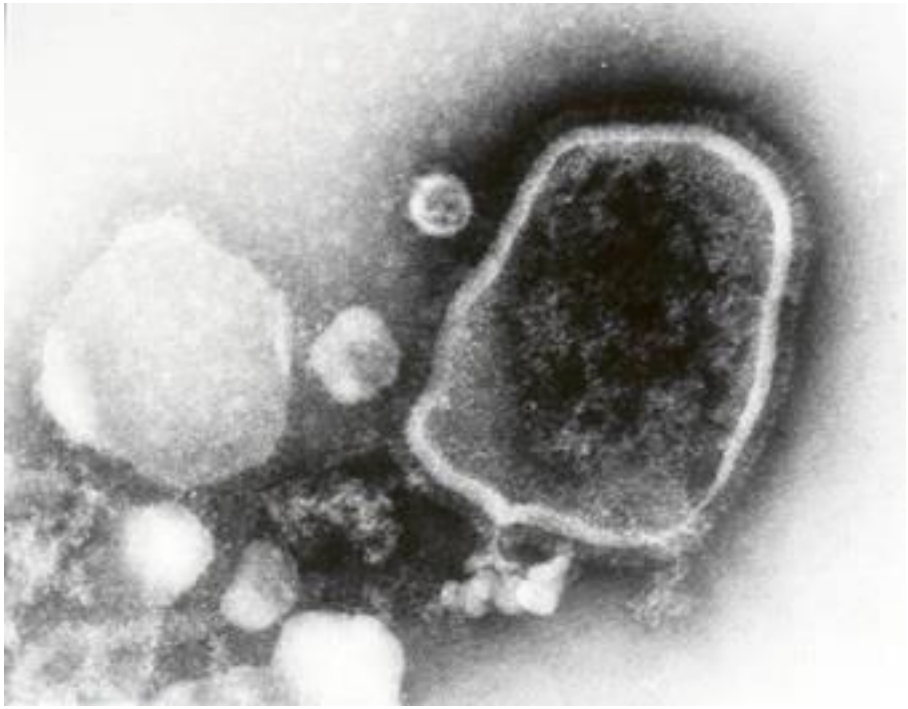
Indirect

- Advantages:
  - Detection of past infections possible
- Disadvantages:
  - Earliest time point 7 days after infection
  - No simple differentiation between acute or past infection

Direct: Electron microscopy



Rotavirus



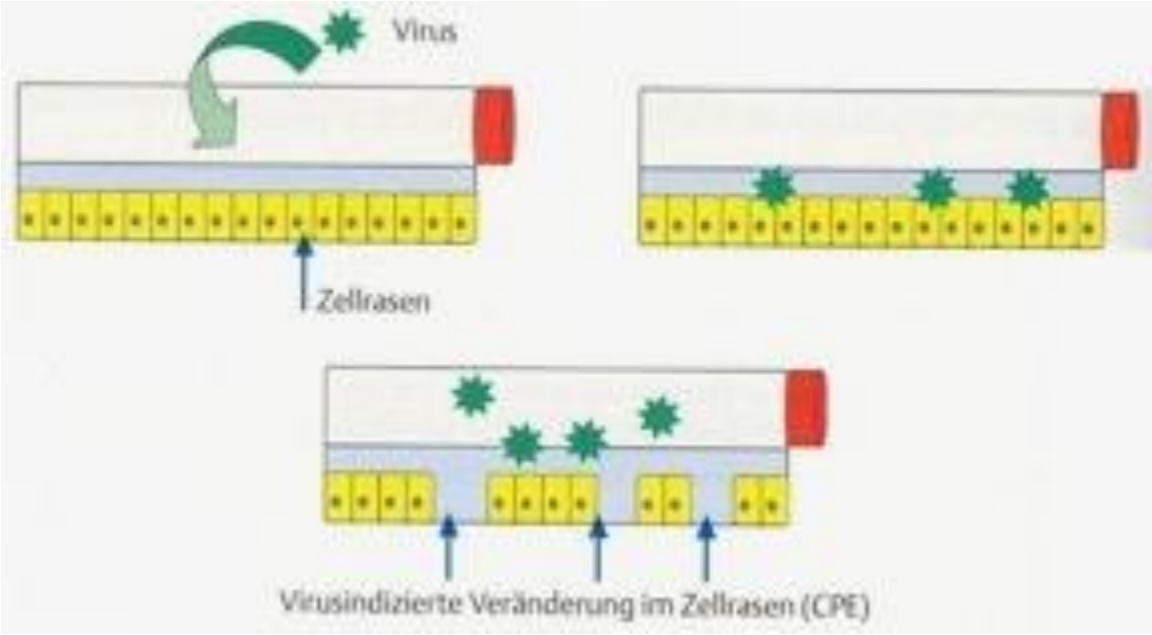
Pleomorph RSV particles

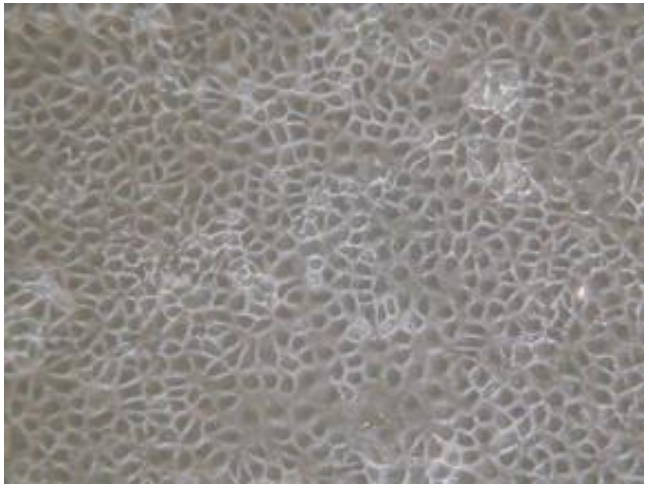
Direct: Electron microscopy

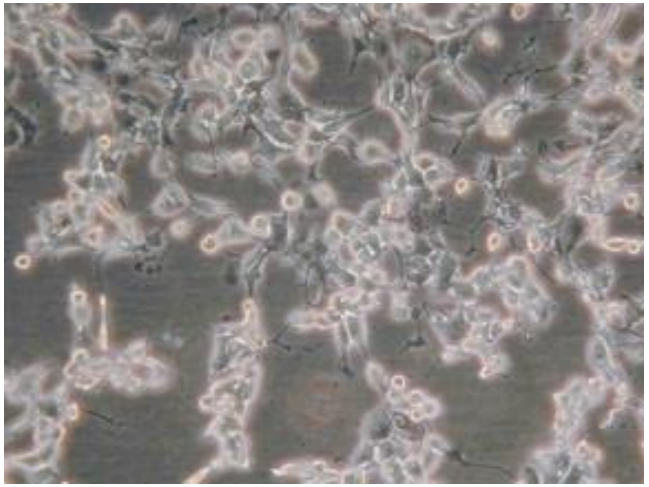
- Advantages:
  - Viruses can be identified as a potential pathogen, when present (SARS)
  - Novel viruses (structure) can be characterized without prior knowledge

- Problems:
  - Laborious
  - Expensive
  - Even professionals can not identify all virsuses from morphology (SARS was beleived to be a Paramyxovirus from EM, but was later identified as a Coronavirus)

Direct: Virus isolation in cell culture







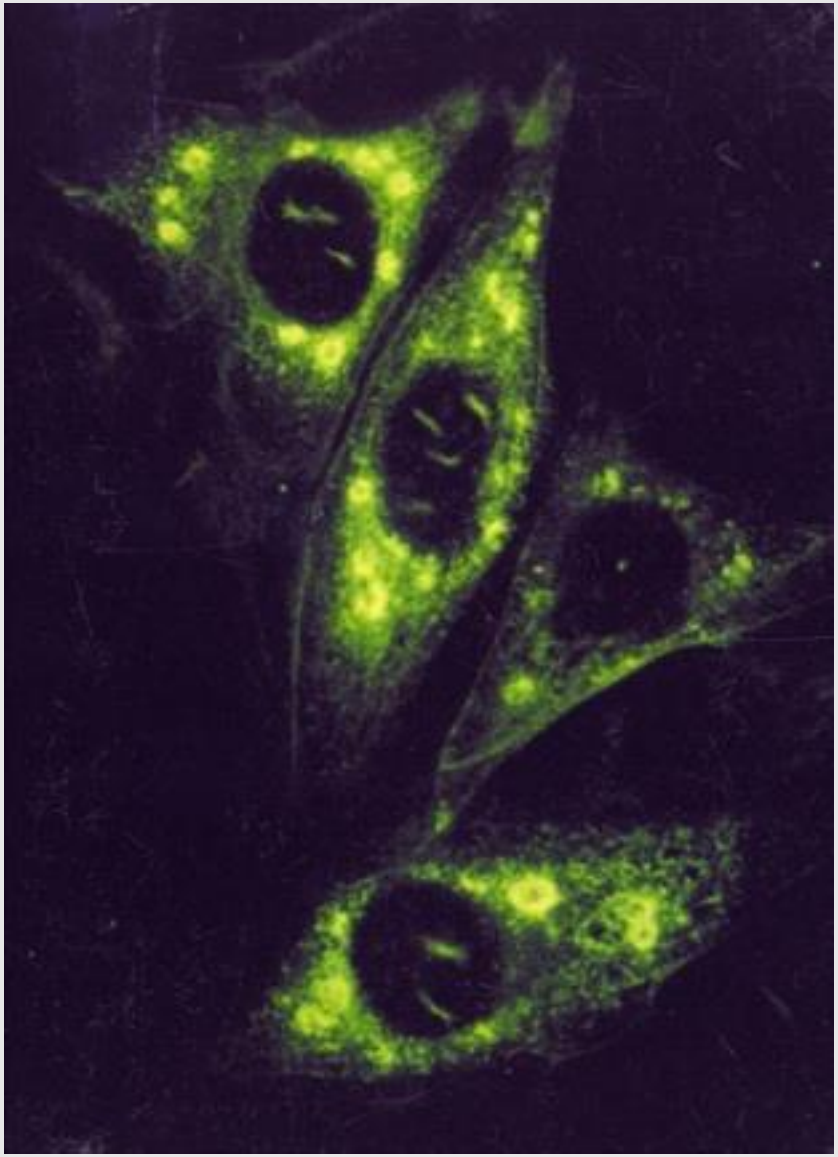
Direct: Virus isolation in cell culture

- Advantage:
  - Sensitive
  - Unknown viruses or strains can be investigated in more detail

- Problems:
  - Laborious and time consuming (days to weeks)
  - Expensive
  - Only presence of „some“ virus can be shown without further experiments

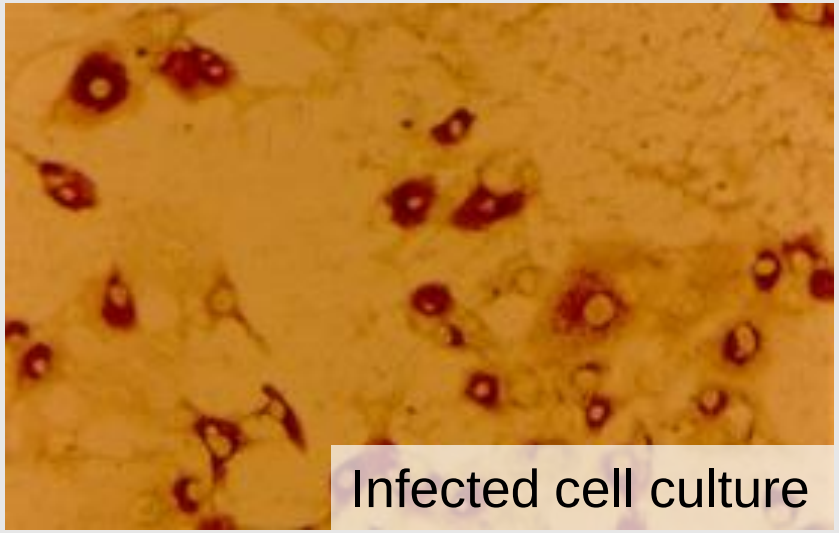
Direct: Direct Immuno fluorescence

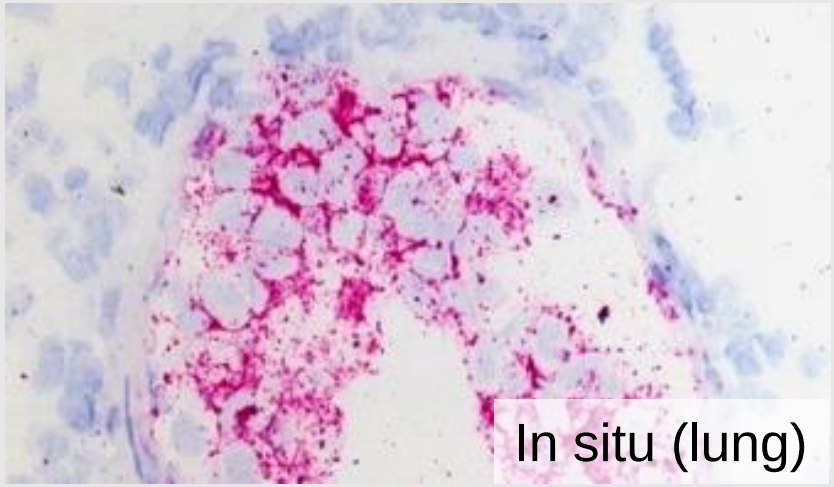
- Sample cells from patient
- Permeabilization of cell membrane: alcohol/detergence
- Incubation with antigen-specific antibody
- Wash steps
- Fluorophore-conjugated secondary antibody (FITC)
- Wash steps
- Analysis under fluorescence microscope



Direct: Immuno (histo-) chemistry

- Infection of cell culture
- Permeabilization of cell membrane: alcohol/detergence
- Incubation with antigen-specific antibody
- Wash steps
- **Enzyme-conjugated secondary antibody (HRP/AP)**
- Wash steps
- **Substrate reaction**
- **Analysis under light microscope**


Infected cell culture


In situ (lung)

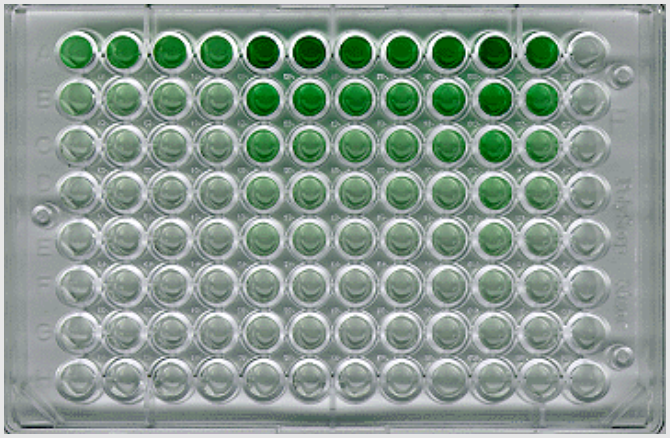
Direct: Immuno fluorescence/(histo-) chemistry

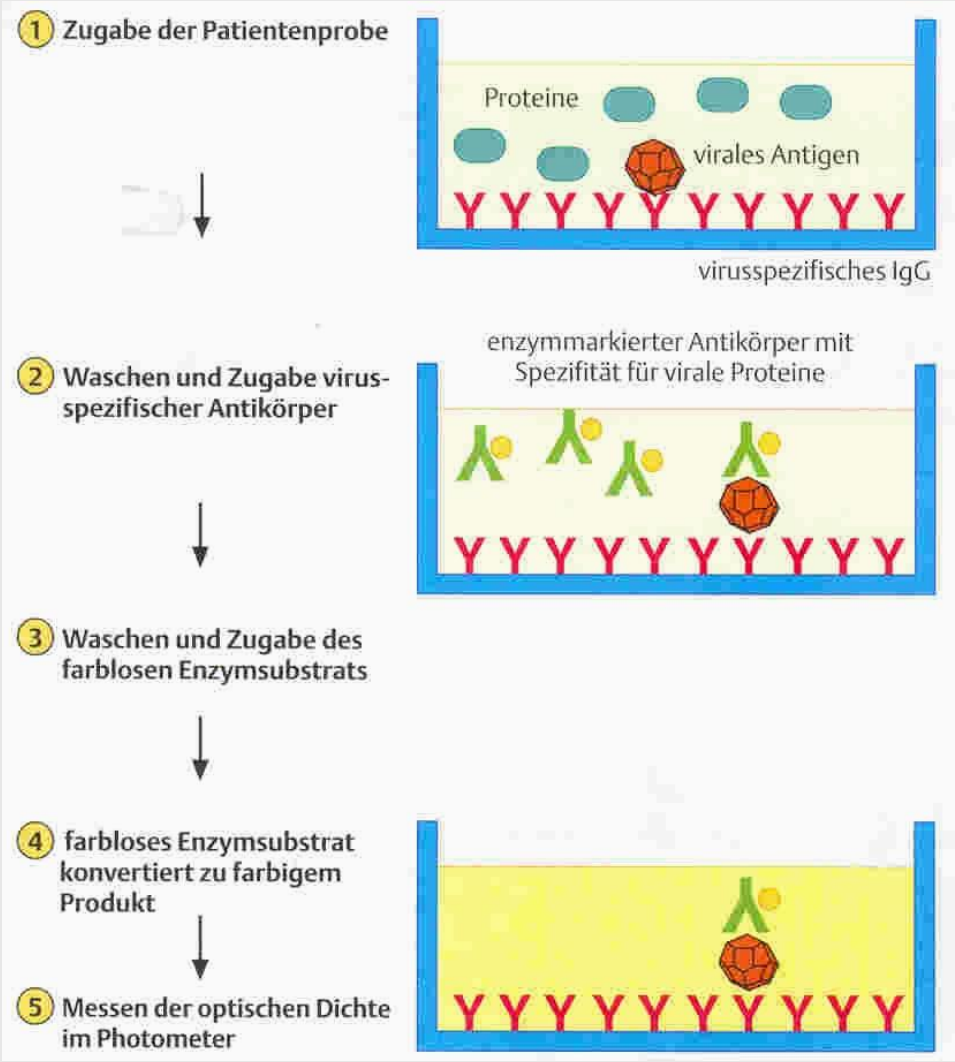
- Advantage:
  - Fast (< 2 hours)
  - Very specific

- Problems:
  - Few samples feasible/available

Direct: Antigen ELISA (enzyme linked immuno sorbent assay)

- Microtiter plate well with immobilized catcher antibody
- Addition of sample (antigen)
- Wash step
- Enzyme-conjugated antibody
- Wash step
- Substrate reaction



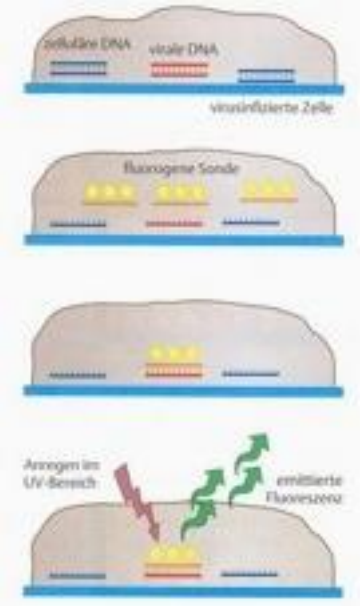


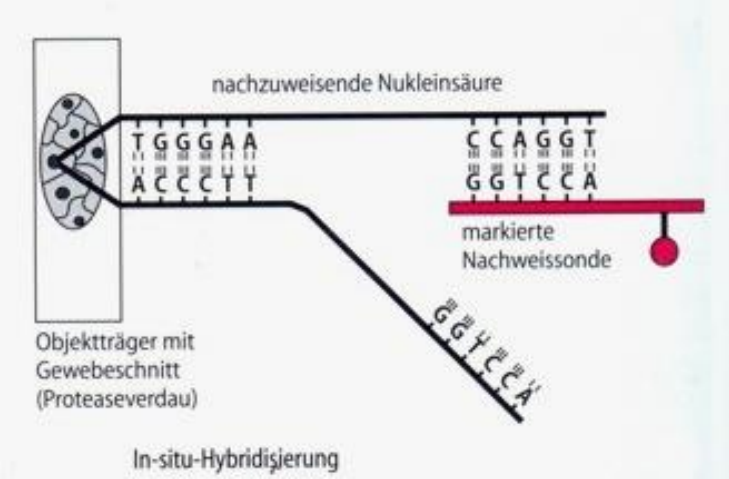
Direct: Antigen ELISA (enzyme linked immuno sorbent assay)

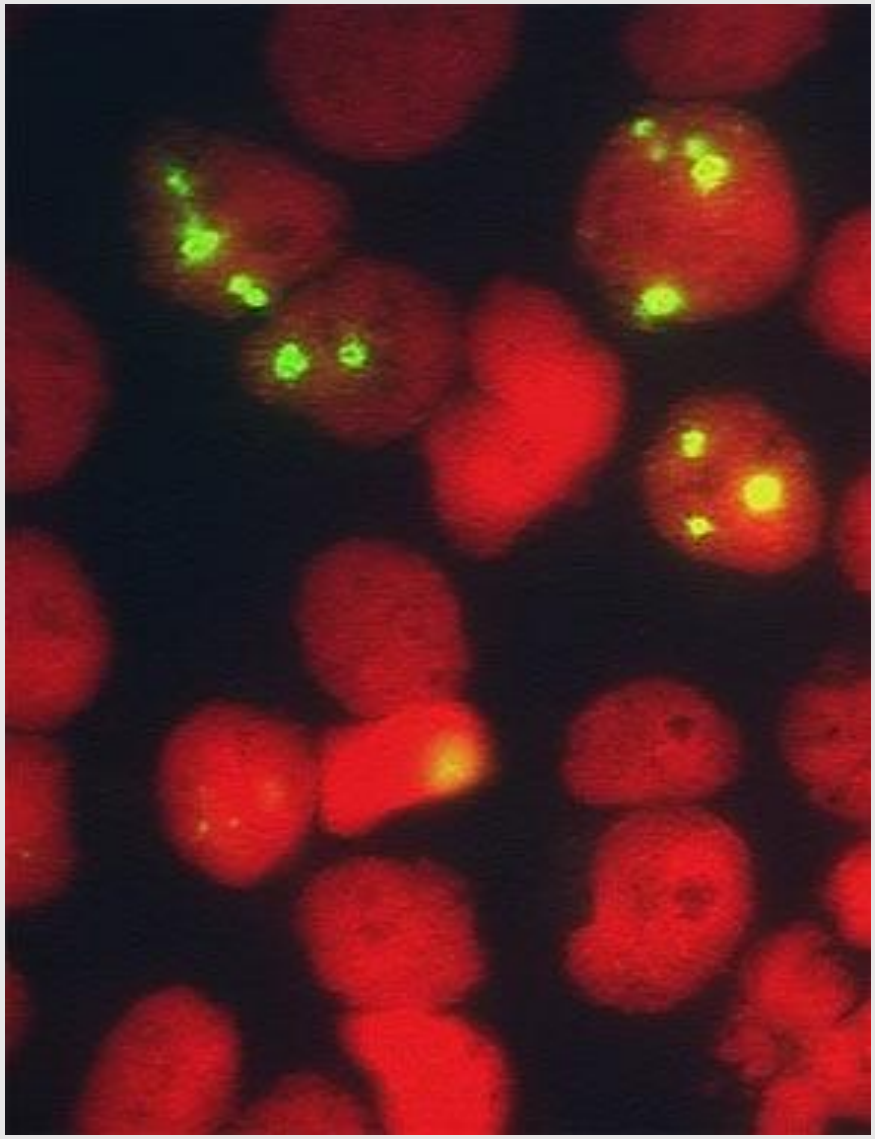
- Advantage:
  - Fast (< 2 hours)
  - Cheap
- Problems:
  - Low sensitivity

Direct: In situ hybridization

- Fixation, permeabilization, denaturation of na
- Fluorescently labeled probe
- Hybridization, Wash
- Analyis under fluorescence microscope







Direct: In situ hybridization

- Advantage:
  - Detection of latent infections

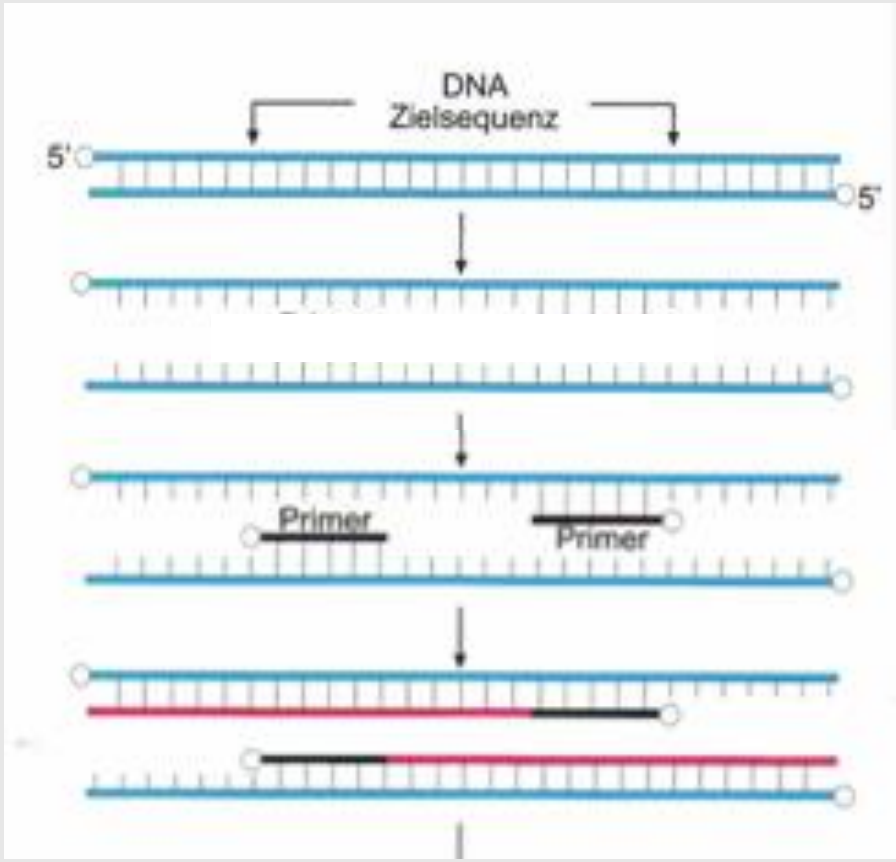
- Problems:
  - Laborious
  - Expensive

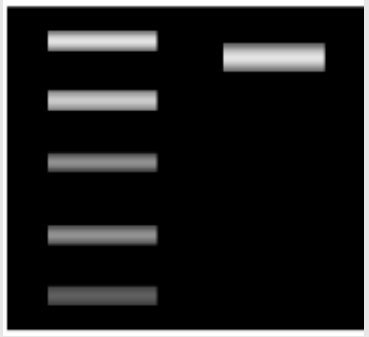
Direct: PCR

1. Denaturation (95 °C)

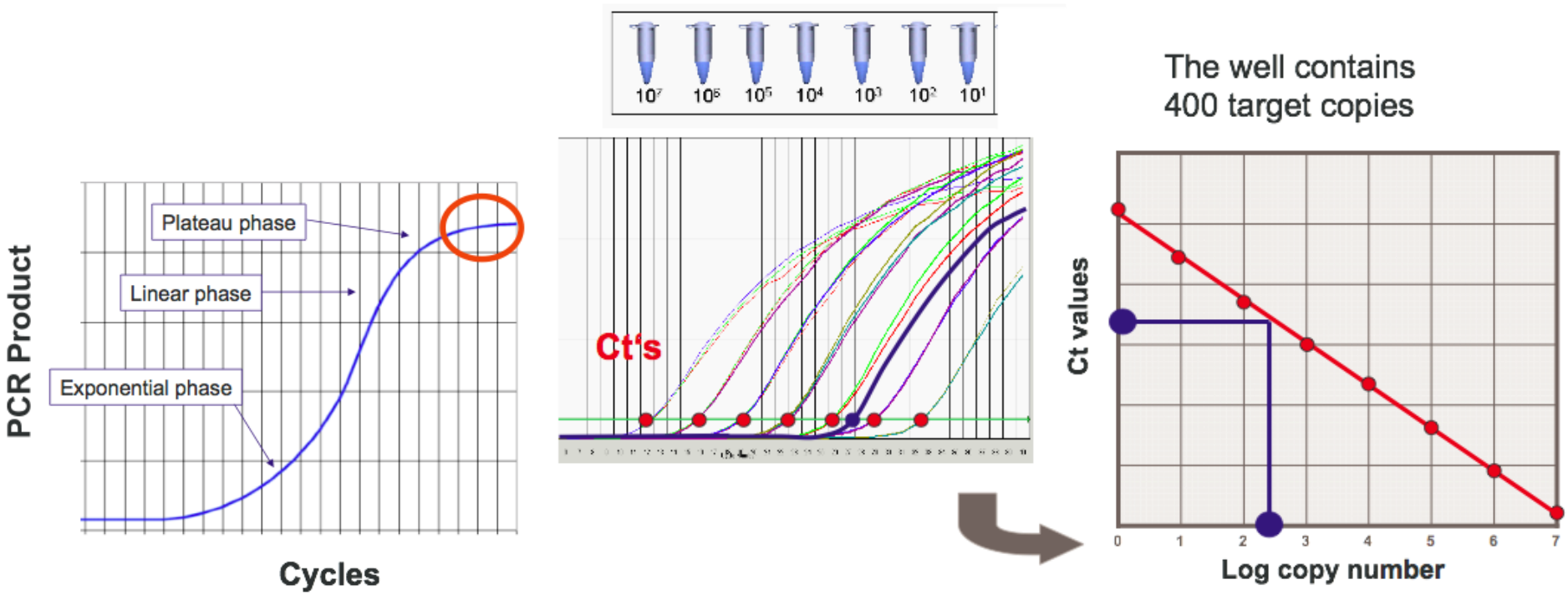
2. Primer annealing (~ 52-68 °C)

3. Primer extension (72 °C)



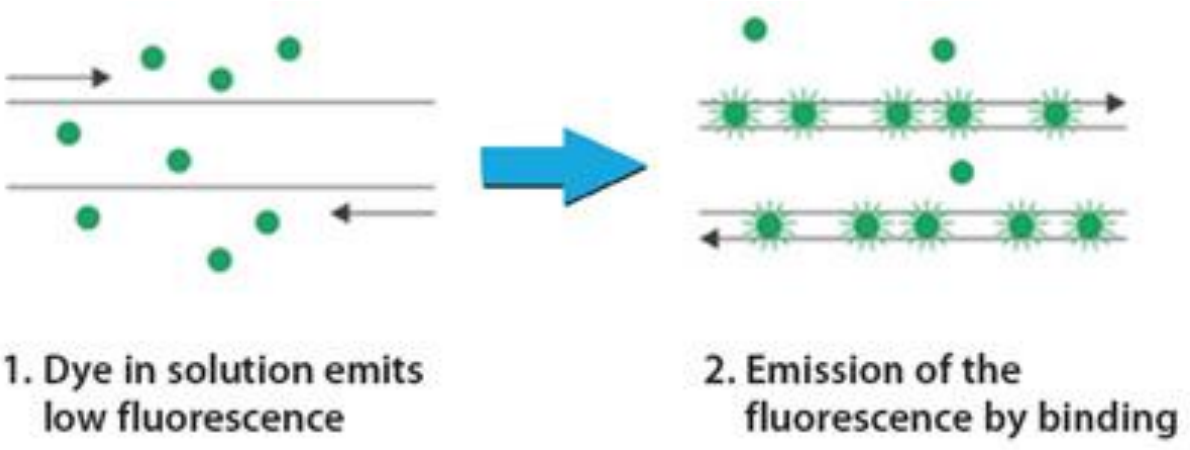


Direct: Real-Time PCR (quantitative PCR)



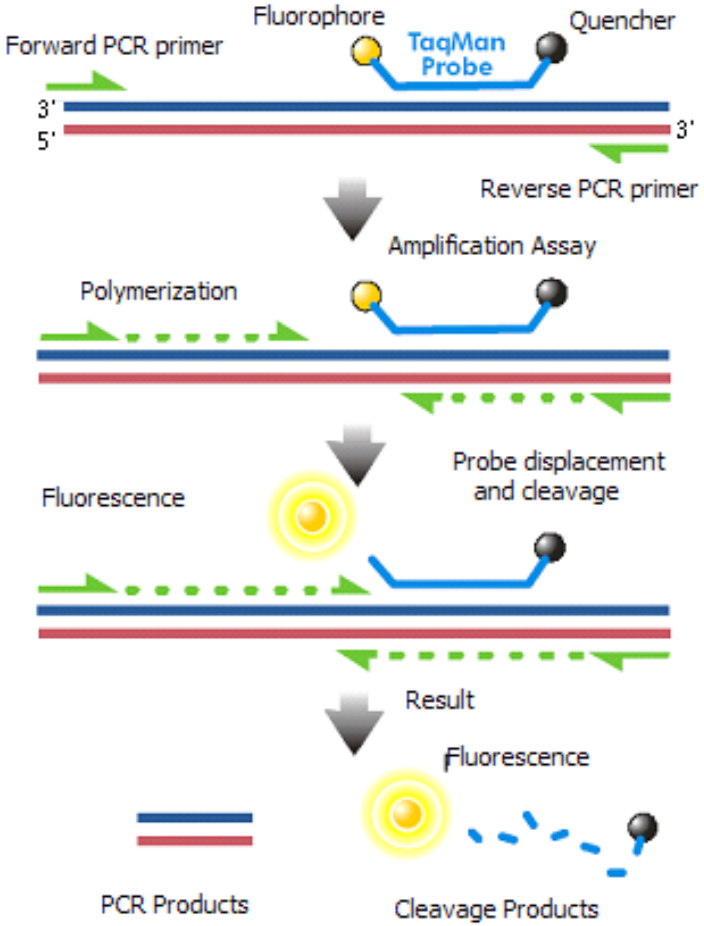
Direct: Real-Time PCR (quantitative PCR)

Sybr Green



Direct: Real-Time PCR (quantitative PCR)

TaqMan Probe

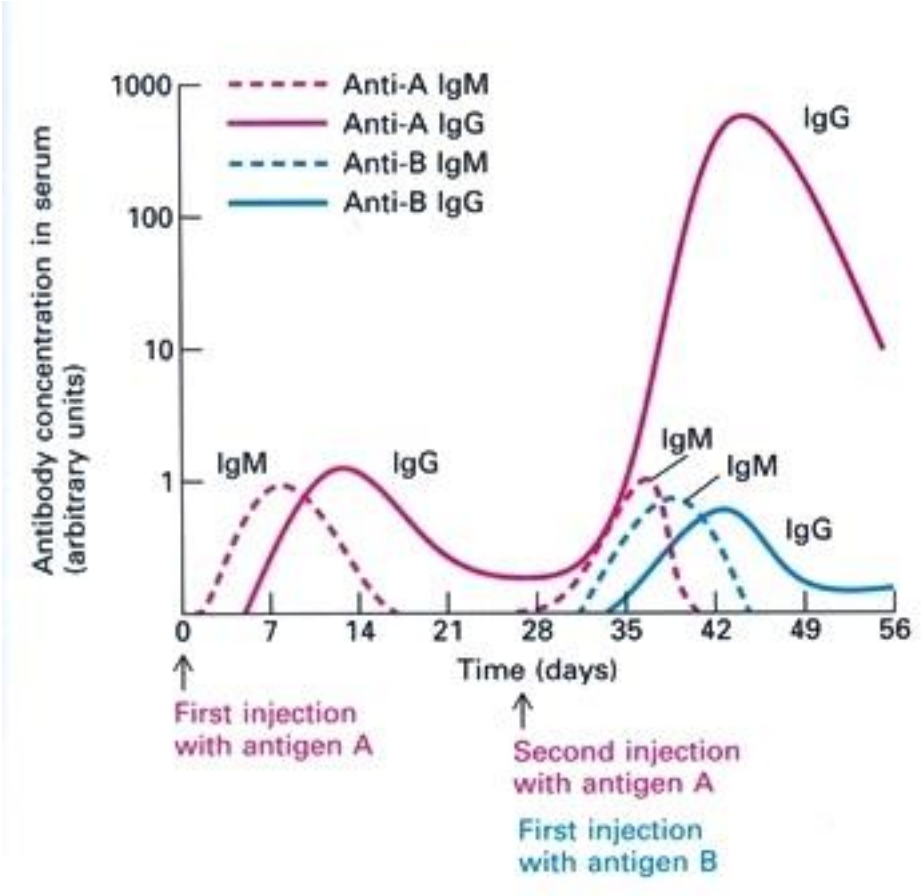


Direct: PCR

- Advantage:
  - Extremely sensitive and specific
  - Early detection of infection possible

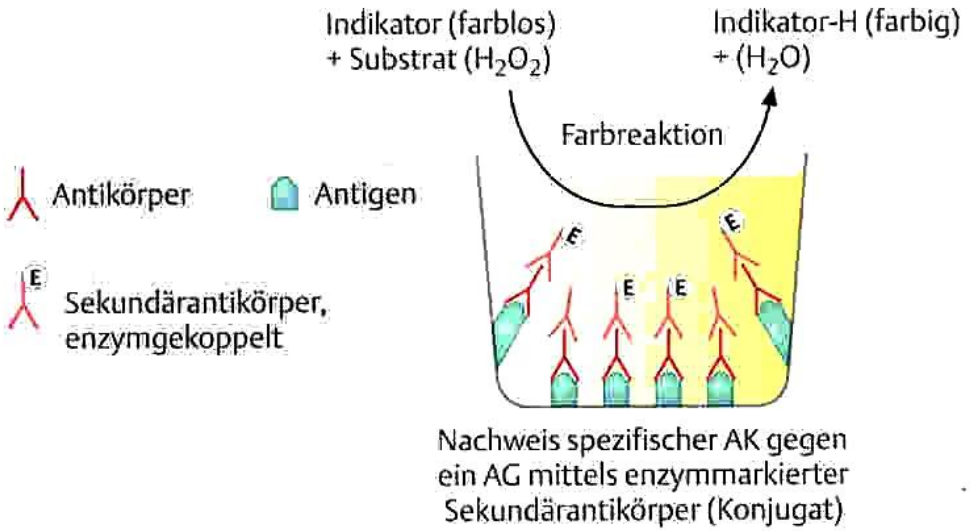
- Problems:
  - Expensive
  - time-consuming
  - Positive results of sometimes no clinical relevance (viral latency)

Indirect: Serological Assays

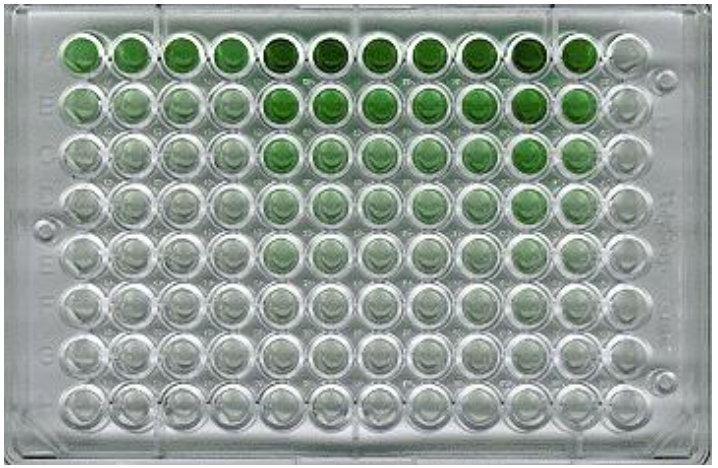


- Detection of IgM
- Detection of IgG
- Increase of IgG-Titer within 10-14 days

Indirect: Antibody ELISA



Nachweis spezifischer AK gegen ein AG mittels enzymmarkierter Sekundärantikörper (Konjugat)



- Microtiter plate well with immobilized antigen
- Addition of sample (antibody)
- Wash step
- Enzyme-conjugated antibody
- Wash step
- Substrate reaction

- Serial Dilution of patient‘s sample (1:2)
- Highest dilution with color value above negative control = titer
- Alternatively: standards of known titers in parallel

Indirect: Antibody ELISA

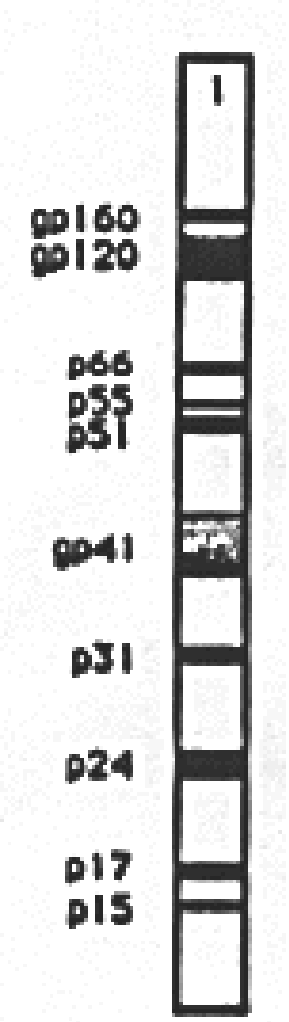
- Advantage:
  - Fast (< 2 hours)
  - Cost-efficient
  - Sensitive
  - Differentiation between antibody class (IgG, IgM) possible

- Problems:
  - All antibodies are detected (neutralizing or only binding)
  - -> no information about protection
  - Earliest time point 1 week after infection
  - Problem of false-positive results (cross-reacting antibodies)

Indirect: Western Blot with serum sample

- Commercially available:
  - Viral antigens are seperated by SDS-PAGE
  - „Western-Blotted“ on nitrocellulose membrane
- Incubation with patient serum
- Detection of antigen specific antibodies by secondary antibody conjugated to an enzyme
- Color reaction

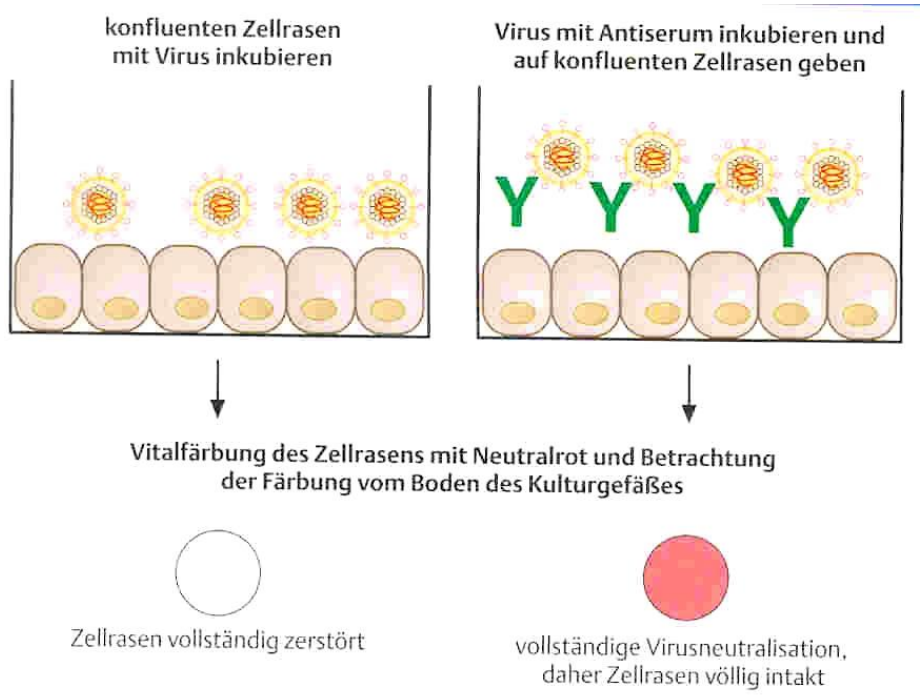
- Typically, antibodies against many antigens are developed at viral infection

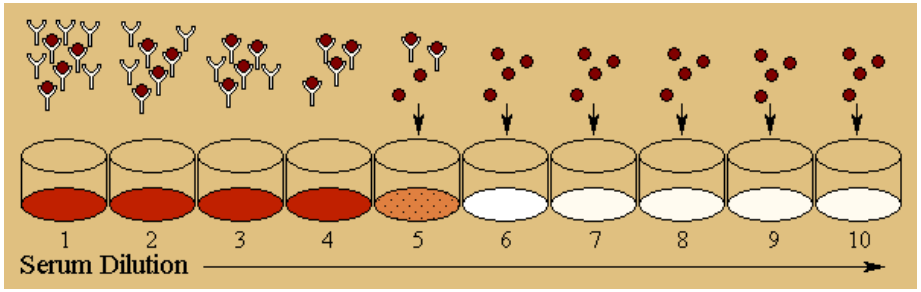


Indirect: Western Blot with serum sample

- Advantage:
  - Highly specific
- Problems:
  - Time-consuming
  - Expensive

Indirect: Virus neutralization assay





- Defined infectious dose of virus
- Preincubation with serum dilution
- Infection of cell culture with preincubated virus/serum

Direct: Virus neutralization assay

- Advantage:
  - binding-only antibodies are not detected
  - Neutralizing antibodies -> measure of protection

- Problems:
  - Time-consuming
  - Expensive

Thank you...